

C.F.R. §1.821(e). As required by 37 C.F.R. §1.825, Applicant's Agent hereby states that the content of the Substitute "Sequence Listing" in paper form and the computer readable form of the Substitute "Sequence Listing" are the same and, as required by 37 C.F.R. §1.825, also states that the submission includes no new matter.

Please amend the application as follows:

In the Specification

*C,* Please replace the "Sequence Listing" filed on December 2, 1996 (pages 118 through 146) with the attached Substitute "Sequence Listing" (sheets 1/25 through 25/25) comprising SEQ ID NOS: 1-67.

Replace the paragraph at page 7, lines 3 through 7 with the following paragraph:

*G,* Figure 7 is an illustration of the nucleotide sequence of a double stranded nucleic acid (coding strand, SEQ ID NO:11; non-coding strand, SEQ ID NO:64) encoding the mouse Act-1 light chain variable region joined to the mouse Act-1 light chain signal peptide sequence, and the deduced amino acid sequence of the Act-1 light chain variable region joined to the mouse Act-1 light chain signal peptide sequence (SEQ ID NO:12).

Replace the paragraph at page 7, lines 8 through 11 with the following paragraph:

*G,* Figure 8 is an illustration of the nucleotide sequence of a double stranded nucleic acid (coding strand, SEQ ID NO: 13; non-coding strand, SEQ ID NO:65) encoding the mature human GM607'CL antibody kappa light chain variable region, and the deduced amino acid sequence of the mature human GM607'CL antibody kappa light chain variable region (SEQ ID NO:8).

Replace the paragraph at page 7, lines 12 through 23 with the following paragraph:

*G,* Figure 9 is an illustration of the nucleotide sequence of a double stranded nucleic acid (coding strand SEQ ID NO:14; non-coding strand, SEQ ID NO: 66) encoding the mouse Act-1 antibody heavy chain and signal peptide, and the deduced amino acid sequence of the mouse Act-

*64*  
*contd*

1 antibody heavy chain variable region and heavy chain signal peptide (SEQ ID NO:15). The nucleotide sequence of the variable region is joined to a nucleotide sequence which encodes a deduced mouse Act-1 heavy chain signal peptide sequence, to yield a composite sequence. (The identity of the primer which amplified the heavy chain region was deduced from the degenerate sequence, and an amino acid sequence for the signal peptide was derived from the primer, downstream sequence and sequences of other signal peptides. The signal peptide shown may not be identical to that of the Act-1 hybridoma.)

Replace the paragraph at page 7, lines 24 through 31 with the following paragraph:

*65*

Figure 10 is an illustration of the nucleotide sequence of a double stranded nucleic acid (coding strand SEQ ID NO:16; non-coding strand, SEQ ID NO: 67) encoding the human 21/28'CL antibody heavy chain and signal peptide, and the deduced amino acid sequence of the human 21/28'CL antibody heavy chain variable region and heavy chain signal peptide (SEQ ID NO:17). The nucleotide sequence encoding the variable region is joined to a nucleotide sequence which encodes a signal peptide sequence derived from the  $V_H$  of human antibody HG3'CL (Rechavi, G., et al., *Proc. Natl. Acad. Sci., USA* 80:855-859 (1983)), to yield a composite sequence.

Please replace Table 3 at pages 53 through 63, with the following Table 3 presented on pages 4-14 of this Amendment:

Table 3. Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V<sub>L</sub> regions.

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Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:7)	Mouse κ-II (SEQ ID NO:50)	Human κ-II (SEQ ID NO:51)	GM 607CL (SEQ ID NO:8)	Act-1 or RH V <sub>k</sub> (SEQ ID NO:52)	Surface or Buried	Comment
1	1	FR1	D	D*	D*	D	D		
2	2		V	V	I*	I	V	buried	Canonical AA for L1 loop ( $\Delta$ 1).
3	3		V	V	V*	V	V		
4	4		V	M	M	M	M	buried	Buried between L1 and L2. V=9/245, M=202/245 in mouse κ-II. M=42/45, V not seen in human κ-II. If binding is poor, consider changing this to V at in second version.
5	5		T	T*	T*	T	T		
6	6		Q	Q*	Q*	Q	Q		
7	7		T	T	S	S	S	surface	Distal to binding site (BS). T=164/245 in mouse κ-II. T=10/37, S=27/37 in human κ-II.
8	8		P	P	P*	P	P		
9	9		L	L	L*	L	L		

Table 3. Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V<sub>L</sub> regions (Cont')

Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:7)	Mouse κ-II (SEQ ID NO:50)	Human κ-II (SEQ ID NO:51)	GM 607CL (SEQ ID NO:8)	Act-1 or RH V <sub>k</sub> (SEQ ID NO:52)	Surface or Buried	Comment
10	10	—	S	S	S*	S	S		
11	11	—	L	L	L*	L	L		
12	12	—	P	P	P	P	P		
13	13	—	V	V*	V*	V	V		
14	14	—	S	S	T*	T	T	surface	Distal to BS. S=151/248 in mouse κ-II. T alone (30/30) seen in human κ-II.
15	15	—	F	L	P	P	P	surface	Distal to BS. F=9/253 in mouse κ-II, F not seen in human κ-II. P=29/31 in human κ-II.
16	16	—	G	G*	G*	G	G		
17	17	—	D	D	E	E	E	surface	Distal to BS. E=18/30, D not seen in human κ-II.
18	18	—	Q	Q	P*	P	P	surface	Distal to BS and on a turn. P alone (31/31) seen in human κ-II.

Table 3. Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V<sub>L</sub> regions (Cont').

Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:7)	Mouse κ-II (SEQ ID NO:50)	Human κ-II (SEQ ID NO:51)	GM 607CL (SEQ D NO:8)	Act-1 or RH V <sub>k</sub> (SEQ ID NO:52)	Surface or Buried	Comment
19	19		V	A	A*	A	A	buried	Pointing into core, but standard mouse to human change. V=66/253, A=187/253 in mouse κ-II. A alone (30/30) seen in human κ-II.
20	20		S	S*	S*	S	S		
21	21		I	I*	I*	I	I		
22	22		S	S*	S*	S	S		
23	23	FR1	C	C*	C*	C	C		
24	24	CDR1	R	R	R	R	R		Canonical AA for L1 loop.
25	25	-	S	S*	S*	S	S		Canonical AA for L1 loop.
26	26	-	S	S*	S*	S	S		Canonical AA for L1 loop.
27	27	--	Q	Q	Q	Q	Q		Canonical AA for L1 loop.
27A	28	--	S	S	S	S	S		Canonical AA for L1 loop.
27B	29		L	L	L*	L	L		Canonical AA for L1 loop.

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Table 3. Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V<sub>L</sub> regions (Cont')

Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:7)	Mouse κ-II (SEQ ID NO:50)	Human κ-II (SEQ ID NO:51)	GM 607 CL (SEQ ID NO:8)	Act-1 or RH V <sub>k</sub> (SEQ ID NO:52)	Surface or Buried	Comment
27C	30	—	A	V	L	L	A		Canonical AA for L1 loop.
27D	31	—	K	H	H		K		Canonical AA for L1 loop.
27E	32	—	S	S	S	S	S		Canonical AA for L1 loop.
27F		—	—	—	X	-	-		
28	33	—	Y	N	D	N	Y		Canonical AA for L1 loop.
29	34	—	G	G*	G	G	G		Canonical AA for L1 loop.
30	35	—	N	N	N	Y	N		Canonical AA for L1 loop.
31	36	—	T	T*	N	N	T		Canonical AA for L1 loop.
32	37	—	Y	Y*	Y*	Y	Y		Canonical AA for L1 loop.
33	38	—	L	L*	L*	L	L		Canonical SS for L1 loop.
34	39	CDR1	S	E	N	D	S		Packing AA. Unusual (117/1365). A, H and N most commonly seen here.

Table 3. Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V<sub>L</sub> regions (Cont')

Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:7)	Mouse κ-II (SEQ ID NO:50)	Human κ-II (SEQ ID NO:51)	GM 607CL (SEQ ID NO:8)	Act-1 or RH V <sub>K</sub> (SEQ ID NO:52)	Surface or Buried	Comment
35	40	FR2	W	W*	W	W	W		
36	41		Y	Y	Y	Y	Y		Packing AA. Most common AA.
37	42		L	L*	L	L	L		
38	43		H	Q*	Q	Q	Q	buried	Packing AA. H is unusual (31/1312). Q is most common AA. (1158/1312). H=6/225, Q=219/225 in mouse κ-II. Q=15/17, H not seen in human κ-II.
39	44		K	K	K	K	K		
40	45	-	P	P*	P	P	P		
41	46	-	G	G*	G*	G	G		
42	47	-	Q	Q*	Q	Q	Q		
43	48	-	S	S*	S	S	S		
44	49	-	P	P*	P*	P	P		Packing AA. Most common AA.
45	50	-	Q	K	Q	Q	Q		

Table 3. Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V<sub>L</sub> regions (Cont')

Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:7)	Mouse κ-II (SEQ ID NO:50)	Human κ-II (SEQ ID NO:51)	GM 607CL (SEQ ID NO:8)	Act-1 or RH V <sub>k</sub> (SEQ ID NO:52)	Surface or Buried	Comment
46	51		L	L	L	L	L		Packing AA. Most common AA.
47	52		L	L*	L	L	L		
48	53		I	I*	I*	I	I		Canonical AA for L2 loop.
49	54	FR2	Y	Y	Y*	Y	Y		
50	55	CDR2	G	K	L	L	G		Canonical AA for L2 loop.
51	56		I	V	V	G	I		Canonical AA for L2 loop.
52	57		S	S*	S*	S	S		Canonical AA for L2 loop.
53	58		N	N	N	N	N		
54	59		R	R	R*	R	R		
55	60		F	F	A	A	F		
56	61	CDR2	S	S*	S*	S	S		
57	62	FR3	G	G*	G*	G	G		
58	63		V	V*	V*	V	V		
59	64		P	P	P*	P	P		
60	65		D	D*	D	D	D		
61	66		R	R*	R	R	R		

Table 3. Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V<sub>L</sub> regions (Cont')

Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:7)	Mouse κ-II (SEQ ID NO:50)	Human κ-II (SEQ ID NO:51)	GM 607CL (SEQ ID NO:8)	Act-1 or RH V <sub>K</sub> (SEQ ID NO:52)	Surface or Buried	Comment
62	67		F	F*	F*	F	F		
63	68		S	S	S*	S	S		
64	69		G	G*	G	G	G		Canonical AA for L2 loop.
65	70		S	S*	S*	S	S		
66	71		G	G*	G*	G	G		
67	72		S	S*	S	S	S		
68	73		G	G*	G	G	G		
69	74		T	T*	T*	T	T		
70	75		D	D	D	D	D		
71	76		F	F*	F*	F	F		Canonical AA for L1 loop.
72	77		T	T*	T*	T	T		
73	78		L	L*	L*	L	L		
74	79		K	K	K	K	K		
75	80		I	I*	I*	I	I		
76	81		S	S	S	S	S		

Table 3. Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V<sub>L</sub> regions (Cont')

Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:7)	Mouse κ-II (SEQ ID NO:50)	Human κ-II (SEQ ID NO:51)	GM 607CL (SEQ ID NO:8)	Act-1 or RH V <sub>κ</sub> (SEQ ID NO:52)	Surface or Buried	Comment
77	82		T	R*	R	R	R	surface	Distal to BS. T=6/221, R=211/221 in mouse κ-II. R=11/12, T not seen in human κ-II.
78	83		I	V	V*	V	V	buried	Pointing into core, but standard mouse to human change. I=6/213, V=195/213 in mouse κ-II. V alone (12/12) seen in human κ-II.
79	84		K	E	E	E	E	surface	Distal to BS. K=20/215, E=191/215 in mouse κ-II. E=9/12, K not seen in human κ-II.
80	85		P	A*	A	A	A	surface	Distal to BS. P=6/183, A=175/183 in mouse κ-II. P=1/12, A=11/12 in human κ-II.

Table 3. Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V<sub>L</sub> regions (Cont')

Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:7)	Mouse κ-II (SEQ ID NO:50)	Human κ-II (SEQ ID NO:51)	GM 607CL (SEQ ID NO:8)	Act-1 or RH V <sub>κ</sub> (SEQ ID NO:52)	Surface or Buried	Comment
81	86		E	E*	E	E	E		
82	87		D	D*	D	D	D		
83	88		L	L	V*	V	V	half buried	Dital to BS. V alone (12/12) seen in human κ-II.
84	89		G	G*	G*	G	G		
85	90		M	V	V*	V	V	half buried	Distal to BS. M=6/212, V=196/212 in mouse κ-II. V alone (12/12) seen in human κ-II.
86	91		Y	Y*	Y*	Y	Y		
87	92		Y	Y	Y*	Y	Y		Packing AA. Most common AA.
88	93	FR3	C	C*	C*	C	C		
89	94	CDR3	L	F	M*	M	L		Packing AA. L is unusual (93/1238). Q is most common AA (654/1238).
90	95		Q	Q*	Q	Q	Q		Canonical AA for L3 loop.

Table 3. Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V<sub>L</sub> regions (Cont')

Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:7)	Mouse κ-II (SEQ ID NO:50)	Human κ-II (SEQ ID NO:51)	GM 607CL (SEQ ID NO:8)	Act-1 or RH V <sub>k</sub> (SEQ ID NO:52)	Surface or Buried	Comment
91	96	—	G	A	A	G			Canonical for L3/Packing AA. 3 <sup>rd</sup> most common AA.
92	97	—	T	T	L	T			Canonical AA for L3 loop.
93	98	—	H	H	Q	H			Canonical AA for L3 loop.
94	99	—	Q	V	X	T	Q		Canonical AA for L3 loop.
95	100	—	P	P*	P	P	P		Canonical AA for L3 loop.
95A		—		P	R*		-		
95B		—		-	-		-		
95C		—		-	-		-		
95D		—		-	-		-		
95E		—		-	-		-		
95F		—		-	-		-		
96	101	—	Y	Y	X	Q	Y		Packing AA. 2 <sup>nd</sup> most common AA.
97	102	CDR3	T	T*	T*	T	T		Canonical for L3.

Table 3. Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V<sub>L</sub> regions (Cont')

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Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:7)	Mouse κ-II (SEQ ID NO:50)	Human κ-II (SEQ ID NO:51)	GM 607CL (SEQ ID NO:8)	Act-1 or RH V <sub>κ</sub> (SEQ ID NO:52)	Surface or Buried	Comment
98	103	FR4	F	F*	F*	F	F		Packing AA. Most common AA.
99	104	-	G	G*	G*	G	G		
100	105	-	G	G	Q	Q	Ω	half buried	Distal to BS. Q=12/13, G=1/12 in human κ-II.
101	106	-	G	G*	G*	G	G		
102	107	-	T	T*	T*	T	T		
103	108	-	K	K*	K	K	K		
104	109	-	L	L*	V	V	Υ	half buried	Distal to BS. L=5/14, V=9/14 in human κ-II.
105	110	-	E	E*	E	E	E		
106	111	-	I	I	I*	I	I		
106A		-	-	-	-	-	-		
107	112	FR4	K	K*	K	K	K		

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Please replace Table 4 at pages 65 through 77, with the following Table 4 presented on pages 16-28 of this Amendment.

Table 4. Alignment of amino acid sequences used in the design of reshaped human mAb Act-1  $V_h$  regions.

Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:9)	Mouse IIB (SEQ ID NO:53)	Human I (SEQ ID NO:54)	human donor 21/28CL (SEQ ID NO:10)	Act-1 RH $V_h$ (SEQ ID NO:55)	Surface or Buried	Comment
1	1	FR1	Q	Q	Q	Q	Q	Q	
2	2	-	V	V*	V	V	V	V	
3	3	-	Q	Q*	Q*	Q	Q	Q	
4	4	-	L	L*	L*	L	L	L	
5	5	-	Q	Q	V	V	V	V	surface
6	6	-	Q	Q	Q	Q	Q	Q	Distal to binding site (BS). Q=135/143 in mouse IIB. V=49/53, Q=1/53 in human I.
7	7	-	P	P	S*	S	S	S	half buried P=102/150 in mouse IIB. P not seen in human I.
8	8	-	G	G*	G*	G	G	G	
9	9	-	A	A	A	A	A	A	
10	10	-	E	E	E	E	E	E	
11	11	-	L	L*	V	V	V	V	surface V=50/54, L= 4/54 in human I

Table 4. Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V<sub>H</sub> regions (Cont')

Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:9)	Mouse IIB (SEQ ID NO:53)	Human I (SEQ ID NO:54)	human donor 2I/28CL (SEQ ID NO:10)	Act-1 RH V <sub>H</sub> (SEQ ID NO:55)	Surface or Buried	Comment
12	12		V	V*	K	K	K	buried	Pointing into core, but standrd mouse to human change. K=41/55, V=3/55 in human I.
13	13		K	K	K*	K	K		
14	14		P	P*	P*	P	P		
15	15		G	G*	G*	G	G		
16	16		T	A	A	A	A	surface	Distal to BS. T=12/139, A=117/139 in mouse IIB. T=1/52, A=23/52 in human I.
17	17		S	S*	S*	S	S		
18	18		V	V*	V	V	V		
19	19		K	K	K	K	K		

Table 4. Alignment of amino acid sequences used in the design of reshaped human mAb Act-1  $V_h$  regions (Cont')

Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:9)	Mouse IIB (SEQ ID NO:53)	Human I (SEQ ID NO:54)	human donor 21/28CL (SEQ ID NO:10)	Act-1 RH $V_h$ (SEQ ID NO:55)	Surface or Buried	Comment
20	20		L	L	V	V	Y	buried	Pointing into core, but standard mouse to human change. L=138/179 in mouse IIB. V=36/52, L=1/52 in human I.
21	21		S	S*	S	S	S		
22	22		C	C*	C	C	C		
23	23		K	K*	K	K	K		
24	24	-	G	A*	A	A	G	buried	Canonical AA for H1 loop ( $\Delta$ 1). G not seen in mouse IIB. G=12/51, A=34/51 in human I.
25	25		Y	S*	S*	S	S	surface	Pointing away from BS and so does not appear to bind antigen (Ag). Y=1/185 in mouse IIB. S=48/50, Y not seen in human I.

Table 4. Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V<sub>H</sub> regions (Cont')

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Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:9)	Mouse IIB (SEQ ID NO:53)	Human I (SEQ ID NO:54)	human donor 21/28CL (SEQ ID NO:10)	Act-1 RH V <sub>H</sub> (SEQ ID NO:55)	Surface or Buried	Comment
26	26		G	G*	G*	G	G		Canonical AA for H1 loop.
27	27		Y	Y	Y	Y	Y		Canonical AA for H1 loop.
28	28		T	T	T	T	T		Canonical AA for H1 loop.
29	29		F	F*	F*	F	F		Canonical AA for H1 loop.
30	30	FR1	T	T	T	T	T		Canonical AA for H1 loop.
31	31	CDR1	S	S	S	S	S		Canonical AA for H1 loop.
32	32		Y	Y	Y	Y	Y		Canonical AA for H1 loop.
33	33		W	W	A	A	W		
34	34		M	M	I	M	M		Canonical AA for H1 loop.
35	35		H	H	S	H	H		Packing AA. Most common AA.
35A		-	-	-	-	-	-		
35B		CDR1	-	-	-	-	-		
36	36	FR2	W	W*	W*	W	W		

Table 4. Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V<sub>H</sub> regions (Cont')

Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:9)	Mouse IIB (SEQ ID NO:53)	Human I (SEQ ID NO:54)	human donor 21/28CL (SEQ ID NO:10)	Act-1 RH V <sub>H</sub> (SEQ ID NO:55)	Surface or Buried	Comment
37	37		V	V	V	V	V		Packing AA. Most common AA.
	38		K	R*	R	R	buried	Pointing into core, but standard mouse to human change. K=177/188 in mouse IIB. R=48/49, K not seen in human I. <i>However, Lys maybe packing H2 loop, therefore consider changing in second version, in conjunction with A40R, if binding poor.</i>	
	39		Q	Q	Q*	Q			Packing AA. Most common AA.

**Table 4.** Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V<sub>H</sub> regions (Cont')

Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:9)	Mouse IIB (SEQ ID NO:53)	Human I (SEQ ID NO:54)	human donor 21/28CL (SEQ ID NO:10)	Act-1 RH V <sub>H</sub> (SEQ ID NO:55)	Surface or Buried	Comment
40	40	—	R	A	A	A	Δ	buried	Pointing into core, but standard mouse to human change. R=160/177 in mouse IIB. A=37/49, R=0/49 in human I. However, Arg <i>maybe packing</i> <i>H2 loop,</i> <i>therefore</i> <i>consider</i> <i>changing in</i> <i>second version, in</i> <i>conjunction with</i> <i>A38K, if binding</i> <i>poor.</i>
41	41	—	P	P	P	P	P		
42	42	—	G	G	G	G	G		
43	43	—	Q	Q	Q	Q	Q		
44	44	—	G	G	G	R	R	buried	Pointing into core, but standard mouse to human change. G=43/48, R=5/48 in human I.

Table 4. Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V<sub>H</sub> regions (Cont')

Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:9)	Mouse IIB (SEQ ID NO:53)	Human I (SEQ ID NO:54)	human donor 21/28CL (SEQ ID NO:10)	Act-1 RH V <sub>H</sub> (SEQ ID NO:55)	Surface or Buried	Comment
45	45	—	L	L*	L*	L	L		Packing AA. Most common AA.
46	46	—	E	E*	E*	E	E		
47	47	—	W	W*	W*	W	W		Packing AA. Most common AA.
48	48	—	I	I*	M	M	I	buried	Ile Underneath and supporting H2 loop ( $\Delta 2$ ). Met=41/48, Ile=1/48 in human I.
49	49	FR2	G	G*	G	G	G		
50	50	CDR2	E	R	W	W	E		
51	51	—	I	I	I	I	I		
52	52	—	D	D	N	N	D		
52A	53	—	P	P*	P	A	P		Canonical AA for H2 loop.
52B	—	—	—	—	Y	—			
52C	—	—	—	—	—	—			
53	54	—	S	N	G	G	S		Canonical AA for H2 loop.

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Table 4. Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V<sub>H</sub> regions (Cont')

Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:9)	Mouse IIB (SEQ ID NO:53)	Human I (SEQ ID NO:54)	human donor 21/28CL (SEQ ID NO:10)	Act-1 RH V <sub>H</sub> (SEQ ID NO:55)	Surface or Buried	Comment
54	55	-	E	S	N	N	E		Canonical AA for H2 loop.
55	56	-	S	G	G	G	S		Canonical AA for H2 loop.
56	57	-	N	G	D	N	N		
57	58	-	T	T	T	T	T		
58	59	-	N	N	N	K	N		
59	60	-	Y	Y	Y	Y	Y		
60	61	-	N	N	A	S	N		
61	62	-	Q	E	Q	Q	Q		
62	63	-	K	K*	K	K	K		
63	64	-	F	F*	F	F	F		
64	65	-	K	K	Q	Q	K		
65	66	CDR2	G	S	G	G	G		
66	67	FR3	K	K*	R	R	R	surface	Distal to BS. R=39/49, K not seen in human I.
67	68	-	A	A*	V	V	V	half buried	Distal to BS. V=45/48, A not seen in human I.
68	69	-	T	T*	T	T	T		

Table 4. Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V<sub>H</sub> regions (Cont')

Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:9)	Mouse IIB (SEQ ID NO:53)	Human I (SEQ ID NO:54)	human donor 21/28CL (SEQ ID NO:10)	Act-1 RH V <sub>H</sub> (SEQ ID NO:55)	Surface or Buried	Comment
69	70		L	L*	I	I	L	buried	Leu Underneath and supporting H2 loop ( $\Delta$ 3). Ile=26/49, Leu=1/49 in human I.
70	71		T	T*	T	T	T		
71	72		V	V	A	R	V	buried	Canonical AA for H2 loop ( $\Delta$ 4).
72	73		D	D*	D	D	D		
73	74		I	K	T	T	I	surface	Behind H2 loop and may play a direct part in Ag binding ( $\Delta$ 5). Ile not seen in mouse IIB or human I. T=21/49 in human I.
74	75		S	S	S*	S	S		
75	76		S	S*	T	A	$\Delta$	surface	Distal to BS. T=26/50, A=4/50, S not seen in human I.
76	77		S	S	S	S	S		
77	78		T	T*	T	T	T		

Table 4. Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V<sub>H</sub> regions (Cont')

Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:9)	Mouse IIB (SEQ ID NO:53)	Human I (SEQ ID NO:54)	human donor 21/28CL (SEQ ID NO:10)	Act-1 RH V <sub>H</sub> (SEQ ID NO:55)	Surface or Buried	Comment
78	79		A	A	A	A	A		
79	80		Y	Y*	Y	Y	Y		
80	81		M	M	M	M	M		
81	82		Q	Q	E	E	E	half buried	Distal to BS. Q=163/194 in mouse IIB. E=35/50, Q=11/50 in human I.
82	83		L	L*	L	L	L		
82A	84		S	S	S	S	S		
82B	85		S	S	S	S	S		
82C	86		L	L*	L*	L	L		
83	87		T	T*	R	R	R	surface	Distal to BS. R=33/51, T=4/51 in human I.
84	88		S	S*	S	S	S		
85	89		E	E	E	E	E		
86	90		D	D*	D*	D	D		

Table 4. Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V<sub>H</sub> regions (Cont')

Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:9)	Mouse IIB (SEQ ID NO:53)	Human I (SEQ ID NO:54)	human donor 21/28CL (SEQ ID NO:10)	Act-1 RH V <sub>H</sub> (SEQ ID NO:55)	Surface or Buried	Comment
87	91		S	S*	T	T	T	surface	Distal to BS. T=48/51, S=2/51 in human I.
88	92		A	A*	A	A	A		
89	93		V	V*	V	V	V		
90	94		Y	Y*	Y*	Y	Y		
91	95		Y	Y	Y	Y	Y		Packing AA. Most common AA.
92	96		C	C*	C*	C	C		Packing AA. Most common AA.
93	97		A	A	A*	A	A		Packing AA. Most common AA.
94	98	FR3	R	R	R	R	R		Canonical AA for H1 loop.
95	99	CDR3	G	Y	A	G	G		Packing AA. 2 <sup>nd</sup> most common residue seen at this point - OK.
96	100		G	Y	P	-	G		
97	101		Y	Y	G	-	Y		
98	102		D	G	Y	-	D		

Table 4. Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V<sub>H</sub> regions (Cont')

Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:9)	Mouse IIB (SEQ ID NO:53)	Human I (SEQ ID NO:54)	human donor 21/28CL (SEQ ID NO:10)	Act-1 RH V <sub>H</sub> (SEQ ID NO:55)	Surface or Buried	Comment
99	103		G	G	G	G	G		
100	104		W	S	S	-	W		
100A	105		D	S	G	-	D		
100B	106		Y	X	G	-	Y		
100C	107		A	X	G	-	A		
100D	108		I	V	C	-	I		Packing AA. I=26/211. F and M are most commonly seen.
100E		-		Y	Y	-			
100F		-		X	R	Y			
100G		-		-	G	Y			
100H		-		Y*	D	G			
100I		-		W	Y	S			
100J		-		Y	X	G			
100K		-		F	F	S			
101	109		D	D	D	N	D		
102	110	CDR3	Y	Y	Y	Y	Y		
103	111	FR4	W	W*	W*	W	W		Packing AA. Most common AA.

Table 4. Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V<sub>H</sub> regions (Cont')

Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:9)	Mouse IIB (SEQ ID NO:53)	Human I (SEQ ID NO:54)	human donor 21/28CL (SEQ ID NO:10)	Act-1 RH V <sub>H</sub> (SEQ ID NO:55)	Surface or Buried	Comment
104	112	-	G	G*	G	G	G		
105	113	-	Q	Q	Q	Q	Q		
106	114	-	G	G*	G*	G	G		
107	115	-	T	T*	T	T	T		
108	116	-	S	T	L	L	L	surface	Distal to BS. T=88/149 in mouse IIB, L=25/39, T=7/39 in human I.
109	117	-	V	V	V*	V	V		
110	118	-	T	T*	T	T	T		
111	119	-	V	V*	V	V	V		
112	120	-	S	S*	S*	S	S		
113	121	FR4	S	S	S*	S	S		

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Table 3. Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V<sub>L</sub> regions (Cont')

Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:7)	Mouse κ-II (SEQ ID NO:50)	Human κ-II (SEQ ID NO:51)	GM 607CL (SEQ ID NO:8)	Act-1 or RH V <sub>k</sub> (SEQ ID NO:52)	Surface or Buried	Comment
91	96	-	G	G	A	A	G		Canonical for L3/Packing AA. 3 <sup>rd</sup> most common AA.
92	97	-	T	T	L	L	T		Canonical AA for L3 loop.
93	98	-	H	H	Q	Q	H		Canonical AA for L3 loop.
94	99	-	Q	V	X	T	Q		Canonical AA for L3 loop.
95	100	-	P	P*	P	P	P		Canonical AA for L3 loop.
95A		-		P	R*		-		
95B		-		-	-		-		
95C		-		-	-		-		
95D		-		-	-		-		
95E		-		-	-		-		
95F		-		-	-		-		
96	101	-	Y	Y	X	Q	Y		Packing AA. 2 <sup>nd</sup> most common AA.
97	102	CDR3	T	T*	T*	T	T		Canonical for L3.

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Table 3. Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V<sub>L</sub> regions (Cont')

Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:7)	Mouse κ-II (SEQ ID NO:50)	Human κ-II (SEQ ID NO:51)	GM 607CL (SEQ ID NO:8)	Act-1 or RH V <sub>κ</sub> (SEQ ID NO:52)	Surface or Buried	Comment
98	103	FR4	F	F*	F*	F	F		Packing AA. Most common AA.
99	104	-	G	G*	G*	G	G		
100	105	-	G	G	Q	Q	Q	half buried	Distal to BS. Q=12/13, G=1/12 in human κ-II.
101	106	-	G	G*	G*	G	G		
102	107	-	T	T*	T*	T	T		
103	108	-	K	K*	K	K	K		
104	109	-	L	L*	V	V	V	half buried	Distal to BS. L=5/14, V=9/14 in human κ-II.
105	110	-	E	E*	E	E	E		
106	111	-	I	I	I*	I	I		
106A	-	-	-	-	-	-	-		
107	112	FR4	K	K*	K	K	K		

Replace Table 4 at pages 65-77 with the below Table 4, presented on pages xv-xxvii and marked up by way of bracketing and underlining to show the changes relative to the previous version of the table.

Table 4. Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V<sub>H</sub> regions.

Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:9)	Mouse IIB (SEQ ID NO:53)	Human I (SEQ ID NO:54)	human donor 21/28CCL (SEQ ID NO:10)	Act-1 RH V <sub>H</sub> (SEQ ID NO:55)	Surface or Buried	Comment
1	1	FR1	Q	Q	Q	Q	Q		
2	2	-	V	V*	V	V	V		
3	3	-	Q	Q*	Q*	Q	Q		
4	4	-	L	L*	L*	L	L		
5	5	-	Q	Q	V	V	V	surface	Distal to binding site (BS). Q=135/143 in mouse IIB. V=49/53, Q=1/53 in human I.
6	6	-	Q	Q	Q	Q	Q		
7	7	-	P	P	S*	S	S	half buried	Distal to BS. P=102/150 in mouse IIB. P not seen in human I.
8	8	-	G	G*	G*	G	G		
9	9	-	A	A	A	A	A		
10	10	-	E	E	E	E	E		
11	11	-	L	L*	V	V	V	surface	Distal to BS. V=50/54, L=4/54 in human I.

Table 4. Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V<sub>H</sub> regions (Cont')

Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:9)	Mouse IIB (SEQ ID NO:53)	Human I (SEQ ID NO:54)	human donor 21/28CL (SEQ ID NO:10)	Act-1 RH V <sub>H</sub> (SEQ ID NO:55)	Surface or Buried	Comment
12	12	-	V	V*	K	K	K	buried	Pointing into core, but standard mouse to human change. K=41/55, V=3/55 in human I.
13	13	-	K	K	K*	K	K		
14	14	-	P	P*	P*	P	P		
15	15	-	G	G*	G*	G	G		
16	16	-	T	A	A	A	A	surface	Distal to BS. T=12/139, A=117/139 in mouse IIB. T=1/52, A=23/52 in human I.
17	17	-	S	S*	S*	S	S		
18	18	-	V	V*	V	V	V		
19	19	-	K	K	K	K	K		

Table 4. Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V<sub>H</sub> regions (Cont')

Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:9)	Mouse IIB (SEQ ID NO:53)	Human I (SEQ ID NO:54)	human donor 21/28CL (SEQ ID NO:10)	Act-1 RH V <sub>H</sub> (SEQ ID NO:55)	Surface or Buried	Comment
20	20		L	L	V	V	V	buried	Pointing into core, but standard mouse to human change. L=38/179 in mouse IIB. V=36/52, L=1/52 in human I.
21	21		S	S*	S	S	S		
22	22		C	C*	C	C	C		
23	23		K	K*	K	K	K		
24	24		G	A*	A	A	G	buried	Canonical AA for H1 loop (Δ1). G not seen in mouse IIB. G=12/51, A=34/51 in human I.
25	25		Y	S*	S*	S	S	surface	Pointing away from BS and so does not appear to bind antigen (Ag). Y=1/185 in mouse IIB. S=48/50, Y not seen in human I.

Table 4. Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V<sub>H</sub> regions (Cont')

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Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:9)	Mouse IIB (SEQ ID NO:53)	Human I (SEQ ID NO:54)	human donor 21/28CL (SEQ ID NO:10)	Act-1 RH V <sub>H</sub> (SEQ ID NO:55)	Surface or Buried	Comment
26	26		G	G*	G*	G	G		Canonical AA for H1 loop.
27	27		Y	Y	Y	Y	Y		Canonical AA for H1 loop.
28	28		T	T	T	T	T		Canonical AA for H1 loop.
29	29		F	F*	F*	F	F		Canonical AA for H1 loop.
30	30	FR1	T	T	T	T	T		Canonical AA for H1 loop.
31	31	CDR1	S	S	S	S	S		Canonical AA for H1 loop.
32	32		Y	Y	Y	Y	Y		Canonical AA for H1 loop.
33	33		W	W	A	A	W		
34	34		M	M	I	M	M		Canonical AA for H1 loop.
35	35		H	H	S	H	H		Packing AA. Most common AA.
35A			-	-	-	-	-		
35B		CDR1	-	-	-	-	-		
36	36	FR2	W	W*	W*	W	W		

Table 4. Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V<sub>H</sub> regions (Cont')

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Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:9)	Mouse IIB (SEQ ID NO:53)	Human I (SEQ ID NO:54)	human donor 21/28CL (SEQ ID NO:10)	Act-1 RH V <sub>H</sub> (SEQ ID NO:55)	Surface or Buried	Comment
37	37		V	V	V	V	V		Packing AA. Most common AA.
38	38		K	R*	R	R	buried	Pointing into core, but standard mouse to human change. K=177/188 in mouse IIB. R=48/49, K not seen in human I. However, Lys maybe packing H2 loop, therefore consider changing in second version, in conjunction with A40R, if binding poor.	
39	39		Q	Q	Q*	Q	Q		Packing AA. Most common AA.

Table 4. Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V<sub>H</sub> regions (Cont')

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Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:9)	Mouse IIB (SEQ ID NO:53)	Human I (SEQ ID NO:54)	human donor 21/28CL (SEQ ID NO:10)	Act-1 RH V <sub>H</sub> (SEQ ID NO:55)	Surface or Buried	Comment
40	40		R	R	A	A	Δ	buried	Pointing into core, but standard mouse to human change. R=160/177 in mouse IIB. A=37/49, R=0/49 in human I. However, Arg maybe packing H2 loop, therefore consider changing in second version, in conjunction with A38K, if binding poor.
41	41		P	P	P	P	P		
42	42		G	G	G	G	G		
43	43		Q	Q	Q	Q	Q		
44	44		G	G	G	R	R	buried	Pointing into core, but standard mouse to human change. G=43/48, R=5/48 in human I.

Table 4. Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V<sub>H</sub> regions (Cont')

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Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:9)	Mouse IIB (SEQ ID NO:53)	Human I (SEQ ID NO:54)	human donor 21/28CL (SEQ ID NO:10)	Act-1 RH V <sub>H</sub> (SEQ ID NO:55)	Surface or Buried	Comment
45	45		L	L*	L*	L	L		Packing AA. Most common AA.
46	46		E	E*	E*	E	E		
47	47		W	W*	W*	W	W		Packing AA. Most common AA.
48	48		I	I*	M	M	I	buried	Ile Underneath and supporting H2 loop ( $\Delta 2$ ). Met=41/48, Ile=1/48 in human I.
49	49	FR2	G	G*	G	G	G		
50	50	CDR2	E	R	W	W	E		
51	51		I	I	I	I	I		
52	52		D	D	N	N	D		
52A	53		P	P*	P	A	P		Canonical AA for H2 loop.
52B		-	-	-	Y	-			
52C		-	-	-	-	-			
53	54		S	N	G	G	S		Canonical AA for H2 loop.

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Table 4. Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V<sub>H</sub> regions (Cont')

Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:9)	Mouse IIB (SEQ ID NO:53)	Human I (SEQ ID NO:54)	Human donor 21/28CL (SEQ ID NO:10)	Act-1 RH V <sub>H</sub> (SEQ ID NO:55)	Surface or Buried	Comment
54	55		E	S	N	N	E		Canonical AA for H2 loop.
55	56		S	G	G	G	S		Canonical AA for H2 loop.
56	57		N	G	D	N	N		
57	58		T	T	T	T	T		
58	59		N	N	N	K	N		
59	60		Y	Y	Y	Y	Y		
60	61		N	N	A	S	N		
61	62		Q	E	Q	Q	Q		
62	63		K	K*	K	K	K		
63	64		F	F*	F	F	F		
64	65		K	K	Q	Q	K		
65	66	CDR <sub>2</sub>	G	S	G	G	G		
66	67	FR3	K	K*	R	R	R		Distal to BS. R=39/49, K not seen in human I.
67	68		A	A*	V	V	V	half buried	Distal to BS. V=45/48, A not seen in human I.
68	69		T	T*	T	T	T		

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Table 4. Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V<sub>H</sub> regions (Cont')

Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NEO:9)	Mouse IIB (SEQ ID NO:53)	Human I (SEQ ID NO:54)	human donor 21/28CL (SEQ ID NO:10)	Act-1 RH V <sub>H</sub> (SEQ ID NO:55)	Surface or Buried	Comment
69	70		L	L*	I	I	L	buried	Leu Underneath and supporting H2 loop ( $\Delta$ 3). Ile=26/49, Leu=1/49 in human I.
70	71		T	T*	T	T	T		
71	72		V	V	A	R	V	buried	Canonical AA for H2 loop ( $\Delta$ 4).
72	73		D	D*	D	D	D		
73	74		I	K	T	T	I	surface	Behind H2 loop and may play a direct part in Ag binding ( $\Delta$ 5). Ile not seen in mouse IIB or human I. T=21/49 in human I.
74	75		S	S	S*	S	S		
75	76		S	S*	T	A	A	surface	Distal to BS. T=26/50, A=4/50, S not seen in human I.
76	77		S	S	S	S	S		
77	78		T	T*	T	T	T		

Table 4. Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V<sub>H</sub> regions (Cont')

Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:9)	Mouse IIB (SEQ ID NO:53)	Human I (SEQ ID NO:54)	human donor 21/28CL (SEQ ID NO:10)	Act-1 RH V <sub>H</sub> (SEQ ID NO:55)	Surface or Buried	Comment
78	79	-	A	A	A	A	A		
79	80	-	Y	Y*	Y	Y	Y		
80	81	-	M	M	M	M	M		
81	82	-	Q	Q	E	E	E	half buried	Distal to BS. Q=163/194 in mouse IIB. E=35/50, Q=11/50 in human I.
82	83	-	L	L*	L	L	L		
82A	84	-	S	S	S	S	S		
82B	85	-	S	S	S	S	S		
82C	86	-	L	L*	L*	L	L		
83	87	-	T	T*	R	R	R	surface	Distal to BS. R=33/51, T=4/51 in human I.
84	88	-	S	S*	S	S	S		
85	89	-	E	E	E	E	E		
86	90	-	D	D*	D*	D	D		

Table 4. Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V<sub>H</sub> regions (Cont')

Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:9)	Mouse IIB (SEQ ID NO:53)	Human I (SEQ ID NO:54)	human donor 21/28CCL (SEQ ID NO:10)	Act-1 RH V <sub>H</sub> (SEQ ID NO:55)	Surface or Buried	Comment
87	91		S	S*	T	T	T	surface	Distal to BS. T=4851, S=251 in human I.
88	92		A	A*	A	A	A		
89	93		V	V*	V	V	V		
90	94		Y	Y*	Y*	Y	Y		
91	95		Y	Y	Y	Y	Y		Packing AA. Most common AA.
92	96		C	C*	C*	C	C		Packing AA. Most common AA.
93	97		A	A	A*	A	A		Packing AA. Most common AA.
94	98	FR3	R	R	R	R	R		Canonical AA for H1 loop.
95	99	CDR <sub>3</sub>	G	Y	A	G	G		Packing AA. 2 <sup>nd</sup> most common residue seen at this point - OK.
96	100		G	Y	P	-	G		
97	101		Y	Y	G	-	Y		
98	102		D	G	Y	-	D		

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Table 4. Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V<sub>H</sub> regions (Cont')

Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:9)	Mouse IIB (SEQ ID NO:53)	Human I (SEQ ID NO:54)	Human donor 21/28CL (SEQ ID NO:10)	Act-1 RH V <sub>H</sub> (SEQ ID NO:55)	Surface or Buried	Comment
99	103	-	G	G	G	G	G		
100	104	-	W	S	S	-	W		
100A	105	-	D	S	G		D		
100B	106	-	Y	X	G	-	Y		
100C	107	-	A	X	G	-	A		
100D	108	-	I	V	C	-	I		Packing AA. I=26/1211. F and M are most commonly seen.
100E		-		Y	Y	-			
100F		-		X	R	Y			
100G		-		-	G	Y			
100H		-		Y*	D	G			
100I		-		W	Y	S			
100J		-		Y	X	G			
100K		-		F	F	S			
101	109	-	D	D	D	N	D		
102	110	CDR <sub>3</sub>	Y	Y	Y	Y	Y	Y	
103	111	FR4	W	W*	W*	W	W	W	Packing AA. Most common AA.

Table 4. Alignment of amino acid sequences used in the design of reshaped human mAb Act-1 V<sub>H</sub> regions (Cont')

Kabat	#	FR or CDR	Mouse Act-1 (SEQ ID NO:9)	Mouse IIB (SEQ ID NO:53)	Human I (SEQ ID NO:54)	human donor 21/28CL (SEQ ID NO:10)	Act-1 RH V <sub>H</sub> (SEQ ID NO:55)	Surface or Buried	Comment
104	112		G	G*	G	G	G		
105	113		Q	Q	Q	Q	Q		
106	114		G	G*	G*	G	G		
107	115		T	T*	T	T	T		
108	116		S	T	L	L	L	surface	Distal to BS. T=88/149 in mouse IIB. L=25/39, T=7/39 in human I.
109	117		V	V	V*	V	V		
110	118		T	T*	T	T	T		
111	119		V	V*	V	V	V		
112	120		S	S*	S*	S	S		
113	121	FR4	S	S	S*	S	S		